

Actomyosin contractility rotates the cell nucleus

Abhishek Kumar

*Mechanobiology Institute and Department of Biological Sciences, NUS, Singapore 117411 and
National Centre for Biological Sciences, TIFR, Bangalore 560065, India*

Ananyo Maitra

Department of Physics, Indian Institute of Science, Bangalore 560012, India

Madhuresh Sumit and G.V. Shivashankar

Mechanobiology Institute and Department of Biological Sciences, NUS, Singapore 117411

Sriram Ramaswamy

*Department of Physics, Indian Institute of Science, Bangalore 560012, India and
TIFR Centre for Interdisciplinary Science, 21 Brundavan Colony,*

Osman Sagar Road, Narsingi, Hyderabad 500 075, India

(Dated: February 26, 2013)

The nucleus of the eukaryotic cell functions amidst active cytoskeletal filaments, but its response to the stresses carried by these filaments is largely unexplored. We report here the results of studies of the translational and rotational dynamics of the nuclei of single fibroblast cells, with the effects of cell migration suppressed by plating onto fibronectin-coated micro-fabricated patterns. Patterns of the same area but different shapes and/or aspect ratio were used to study the effect of cell geometry on the dynamics. On circles, squares and equilateral triangles, the nucleus undergoes persistent rotational motion, while on high-aspect-ratio rectangles of the same area it moves only back and forth. The circle and the triangle showed respectively the largest and the smallest angular speed. We show that our observations can be understood through a hydrodynamic approach in which the nucleus is treated as a highly viscous inclusion residing in a less viscous fluid of orientable filaments endowed with active stresses. Lowering actin contractility selectively by introducing blebbistatin at low concentrations drastically reduced the speed and persistence time of the angular motion of the nucleus. Time-lapse imaging of actin revealed a correlated hydrodynamic flow around the nucleus, with profile and magnitude consistent with the results of our theoretical approach. Coherent intracellular flows and consequent nuclear rotation thus appear to be a generic property that cells must balance by specific mechanisms in order to maintain nuclear homeostasis.

Keywords: Nuclear dynamics— Cell shape— Actomyosin— Rheology— Hydrodynamics of active gels— Active stresses

INTRODUCTION

The nucleus is the largest and stiffest organelle in a eukaryotic cell [1]. It is actively coupled to the dynamic cytoskeleton [2–5] by means of a variety of scaffold proteins: contractile [6] acto-myosin complexes, microtubule filaments constantly undergoing dynamic reorganization, and load bearing intermediate filaments [2–4, 7–10]. The nucleus has been found to translate and rotate during cell migration [11–17]. It is reasonable to suppose that such motions are a result of active processes in the cytoplasm, involving the cytoskeleton and molecular motors [2, 3, 7–10, 12, 14, 17]. The positioning of the nucleus in the cellular environment is critical to many physiological functions such as migration, mitosis, polarization, wound healing, fertilization and cell differentiation [16, 18]. Alterations to nuclear position have been implicated in a number of diseases [16, 18]. Taken together these studies suggest that the mechanical homeostatic balance of nuclear positioning and dynamics is intimately coupled with cellular geometry. While a number of molecular players have been implicated in this context [2–5, 9, 10], the role of actomyosin contractility on nuclear dynamics has not been explored.

In this paper, we show that cell geometry and active stresses are critical components in determining nuclear position and movements. Fibroblast cells (NIH3T3) plated on micro-patterned fibronectin surfaces of varying shapes and aspect ratio were used to assess the effect of geometrical constraint on the translational and rotational movement of the nucleus. Time-lapse imaging revealed a correlation between actin flow patterns and nuclear movement. We show that a hydrodynamic model of oriented filaments endowed with active contractile stresses [19–23], with the nucleus entering only as a passive inclusion, gives rise to the observed organized actin flow and nuclear rotation. While preparing the present work for submission, we became aware of two works [24, 25] with theoretical formulation and predicted behaviours similar to ours. The contexts in which these works are set is different from ours, i.e., the

dynamics of the cell nucleus is not the subject of these papers. In addition, the boundary conditions are different in detail. Reference [24] was in a Taylor-Couette geometry, i.e., there is no medium inside the inner circle, and Reference [25] was in a circular geometry without a central inclusion. Our observations suggest that nuclear rotation and circulating flows are an inherent property of the active cell interior under geometric confinement. That nuclear rotation is not a normally observed feature of cell dynamics suggests that the cell must possess other mechanisms to suppress it. We discuss these towards the end of the paper.

MATERIALS AND METHODS

Cell Culture: NIH3T3 fibroblasts (ATCC) were cultured in low glucose DMEM (Invitrogen) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco, Invitrogen) and 1% Penicillin- Streptomycin (Invitrogen). Cells were maintained at 37°C in incubator with 5% CO₂ in humidified condition. Cells were trypsinized and seeded on fibronectin coated patterned surfaces for 3 hours before staining or imaging. For confocal imaging, low well ibidi non- treated hydrophobic dishes were used. 65,000 cells were seeded on each time on patterned surfaces (with 10,000 patterns) for 30 minutes, after which the non-settled cells were removed and media was re-added in the dishes. Blebbistatin (Invitrogen) were diluted from stock using filtered media. Blebbistatin was used at concentration of 1.25 μ M. This minimizes the effect of any other solvent like DMSO. Microtubule was immunostained using α -tubulin antibody (1:200, Abcam) and Alexa fluor 546 secondary (1:500, Invitrogen) in cell plated on triangular pattern. The nucleus was labeled using Hoechst (1:1000).

Preparation of PDMS Stamps and micro-contact printing: PDMS stamps were prepared from PDMS Elastomer (SYLGARD 184, DOW Corning) and the ratio of curer to precursor used was 1: 10. The curer and precursor were mixed homogeneously before pouring onto the micropatterned silicon wafer. The mixture was degassed in the desiccator for at least 30 minutes to remove any trapped air bubbles and was then cured at 80°C for 2 hours, after which the stamps were peeled off from the silicon wafer. Micropatterned PDMS stamps were oxidized and sterilized under high power in Plasma Cleaner (Model PDC-002, Harrick Scientific Corp) for 4 minutes. 30 μ l of 100 μ g/ml fibronectin solution (prepared by mixing 27 μ l of 1xPBS to 1.5 μ g of 1mg/ml fibronectin and 1.5 μ l of Alexa 647 conjugated fibronectin) was allowed to adsorb onto the surface of each PDMS stamp under sterile condition for 20 minutes before drying by tissue. The PDMS stamp was then deposited onto the surface of a low well non-treated hydrophobic dishes (Ibidi) (for high-resolution imaging) to allow transferring of the micro-features. Subsequently, the stamped dish was inspected under fluorescent microscope to verify the smooth transfer of fibronectin micro-patterns. Surface of sample was then treated with 1ml of 2mg/ml Pluronic F-127 for 2 hours to passivate non- fibronectin coated regions.

Cell Transfection: Transfection of various plasmids in wt NIH3T3 cells was carried out using JetPRIME polyplus transfection kit. 1 μ g of plasmid was mixed properly in 100 μ l of JetPRIME buffer by vortexing and spinning, 3.5 μ l of JetPRIME reagent was then added and the mixture was again vortexed and spun. The mixture was incubated for 30 minutes and then added to 50-60% confluent culture in 35mm dish. Cells were kept in fresh media for 2 hours prior to addition of transfection mixture. Cells were incubated for 20 hrs before plating them on the patterned substrates.

Imaging: Phase contrast imaging of cells on different geometrical patterns was done on Nikon Biostation IMq using 40x objective at 37°C in a humidified chamber with 5% CO₂. Confocal time lapse imaging of cells transfected with various plasmids (Lifeact EGFP, τ RFP and dsRed ER) was carried out on Nikon A1R using 60x, 1.4 NA oil objective at 37°C in a humidified incubator with 5% CO₂.

Image Analysis and quantifications: Acquired images were processed and analysed using ImageJ software (<http://rsbweb.nih.gov/ij/index.html>). To determine the translational coordinates and rotational angle of nucleus, diagonally opposite nucleoli were manually tracked from the phase contrast image of the cell using the ImageJ plugin-MtrackJ (<http://www.imagescience.org/meijering/software/mtrackj/>). The translational and rotational autocorrelation were calculated from the residual of the linear fit to corresponding curves- the detrended curves, thereby taking into account only the time scales relevant in our measurements. Particle image velocimetry (PIV) analysis was carried out using Matlab PIV toolbox-Matpiv between consecutive image frames separated by 1min. Images acquired were 512 X 512 pixels. The size of the interrogation window was chosen to be 32 X 32 with an overlap of 50% between the consecutive time frames. The single pass method was used for calculating the velocities. Quantifications were done

using custom written program in either LabVIEW 6.1 or MATLAB R2010a. All the graphs and curve fittings were carried out using OriginPro 8.1 (OriginLab Corporation, Northampton, USA).

RESULTS AND DISCUSSION

Geometric constraints on the cell affects the dynamics of the nucleus

To assess nuclear dynamics independent of cell migration, we used micro-patterned fibronectin-coated substrates to confine cells to regions of defined geometry and size. Single cells were cultured on each patterned substrate and time lapse phase contrast imaging was carried out for about 8 hours (or till the cell underwent mitosis). Geometries with a variety of rotational symmetries – circle, square, equilateral triangle and rectangles with aspect ratios 1:3 and 1:5 – but the same cell spreading area ($1600\mu\text{m}^2$) – were fabricated and used to study effect of cell shape on the translational and rotational movement of the nucleus. Figure 1A-E shows color-coded intensity-profile images obtained by average-intensity projection of phase-contrast time lapse images for the above cases (Figure 1A-C are for rectangles with aspect ratio 1:1, 1:3 and 1:5, Figure 1D and E are for triangle and circle). The dark color or low intensity at the vertices of the triangular and rectangular patterns shows the formation of stable contacts in that region, a feature absent on the circular pads. Note that the cell adheres much more stably on the triangular pattern than on the circle or the square.

The translational and rotational movements were measured from the time lapse images, and show convincingly the influence of cell geometry on nuclear dynamics. Figure 1F displays typical trajectories of the nucleus on triangular and rectangular geometries of same area. On rectangles, presumably because of narrower confinement, the nucleus undergoes mainly translation while on triangles (as well as circles and squares) the nucleus both rotates and translates, as shown in Supplementary Movie 1-5. Since motion out of plane is negligible, we resolve the dynamics into two-dimensional translation and rotation in the XY plane. Translation is estimated through the instantaneous mean position $(x, y) = (\frac{x_1+y_1}{2}, \frac{x_2+y_2}{2})$ of two nucleoli situated at roughly diametrically opposed points (x_1, y_1) and (x_2, y_2) . The top inset to Figure 1F shows a typical translation trajectory. Rotation is characterised by the coordinates of one nucleolus relative to this mean. A typical rotational track for the nucleus is shown in the bottom inset to Figure 1F. Although rotation of the nucleus is not a normal feature of cell cycle, both translational and rotational movement of the nucleus during cell migration has been reported [11–18] for many cell types including NIH3T3 which was used in all our experiments. For completeness, we document such motion here as well. Figure S1A shows a representative DIC image of a monolayer of NIH3T3 cells cultured on glass bottom dishes. Time lapse images (Figure S1B) of three cells from this field of view are presented with arrows showing the position of the nucleolus. Rotation and translation tracks of the nucleus are plotted for these cells in Figure S1C and D respectively, showing large departures from its initial position and orientation. Finally, in order to demonstrate that migration is not the underlying cause of the rotation we observe, we confine cells by plating them onto fibronectin patterns of various well-defined geometries, allowing us to study the effect of cell geometry alone on nuclear dynamics.

The fraction of rotating nuclei decreases significantly in geometries with large aspect ratio whereas on more symmetric patterns namely equilateral triangles, squares and circles, the fraction is not significantly different (Figure 2A). Except on rectangles, about 80% of cells showed at least 90° nuclear rotations in 8 hours. Nuclear circularity as a function of cell shape is altered with changes in aspect ratio (decreases from 0.9 to 0.7, Supplementary Figure S2A) but not with changes in rotational symmetry of constraints. The instantaneous linear velocity decreases marginally from circle to square to triangle as well as with increase in aspect ratio of rectangle (1:1 - $0.23\mu\text{m}/\text{min}$ and 1:5 - $0.20\mu\text{m}/\text{min}$, see Figure 2B and Supplementary Figure S2B). The mean rotational velocity decreases from circle ($2.2^\circ/\text{min}$) to square ($2.0^\circ/\text{min}$) to triangle ($1.6^\circ/\text{min}$) (Figure 2C and Supplementary Figure S2C, D and E) suggesting that rotation is sensitive to geometric constraints. However, the fraction of nuclei showing significant and systematic rotation is similar for these three shapes. To explore the possible role of myosin induced contractility in these phenomena, we turn now to the active hydrodynamic theory [19–23] of the cell interior.

Active fluid with a central inclusion

We show that cytoplasmic flows produced by acto-myosin contractility are the minimal explanation for the observed rotation of the nucleus. To this end, we turn to the theoretical framework of active hydrodynamics [26–29]. Contractile stresses carried by actomyosin, given an arrangement of filaments compatible with the cell shape imposed by the pads

and the presence of the nucleus as an internal obstacle, lead to organized flows that rotate the nucleus. More detailed propulsive elements, e.g., pushing by microtubules anchored onto the nuclear surface [7, 17], while possibly present in the cell, are not a necessary part of the mechanism. Since the cell in the experiment is stretched, its height is smaller than its dimensions in the plane. We can therefore model the cell as a quasi-two-dimensional film with the hydrodynamics being cut off at a scale proportional to the height. We also assume an axisymmetric cell, and ignore actomyosin treadmilling and the on-off kinetics of the motors. This highly simplified view of the cell still exhibits some key features of the dynamics found in the experiment.

We now present the equations of active hydrodynamics [26–29]. The inner circular region represents the nucleus, which is taken to contain no active motor-filament complexes and is therefore modeled as a passive liquid drop of very high viscosity (η_i) – in effect undeformable. The outer annular region is the cytoplasm, which contains active orientable filaments. The inner fluid-fluid interface, i.e., the boundary between cytoplasm and nucleus, has tangential stress continuity and tangential velocity continuity, and the outer surface, the contact line of cell with pad, has no slip. We assume the filaments preferentially lie parallel to any surface with which they are in contact. In particular, they therefore lie tangent to both the inner and the outer boundaries.

The cytoplasmic medium is taken to consist of filaments suspended in the cytosol of viscosity $\eta \ll \eta_i$. We assume the filaments are in a state of well-formed local orientation whose magnitude does not change so that it can be characterised completely by a unit vector or “director” field, $\mathbf{n}(\mathbf{r})$, [33] at position \mathbf{r} . Associated with the filaments is an active stress $W\mathbf{n}(\mathbf{r})\mathbf{n}(\mathbf{r})$, where the parameter W is a measure of actomyosin activity. The concentration of filaments and myosin is assumed uniform. Fluid flow in the cytoplasm is described by the hydrodynamic velocity field \mathbf{v} . The equations of active hydrodynamics in steady state lead to a dynamic balance between shearing and relaxation of filaments [34],

$$\frac{1}{\Gamma} \delta_{ij}^T \frac{\delta F}{\delta n_j} = -v_j \partial_j n_i + \lambda_{ijk} \partial_k v_j, \quad (1)$$

and force balance, ignoring inertia,

$$\nabla_j \sigma_{ij} = \zeta v_i, \quad (2)$$

with total stress tensor

$$\sigma_{ij} = \frac{\eta}{2} [\nabla_i v_j + \nabla_j v_i] - P \delta_{ij} - W n_i n_j + \lambda_{kij} \frac{\delta F}{\delta n_k} + \sigma_{ij}^0. \quad (3)$$

Here $F = \int d^d x f$, $f = (K/2)(\nabla \mathbf{n})^2$, is the elastic free energy for the director \mathbf{n} , with the Frank elastic constant K , $\delta_{ij}^T = \delta_{ij} - n_i n_j$, $\lambda_{kij} = (1/2)(\delta_{ki}^T n_j - \delta_{kj}^T n_i) + (\lambda/2)(\delta_{ki}^T n_j + \delta_{kj}^T n_i)$ is a flow-orientation coupling, and $\sigma^0 = (\nabla \mathbf{n}) \partial f / \partial (\nabla \mathbf{n})$ is the Ericksen stress. We will work with a completely symmetric stress, σ_{ij}^s , built from σ_{ij} , which will give the same velocity field, due to angular momentum conservation [35, 36].

The coefficient ζ in (2) represents in a z -averaged sense the effects of confinement on the damping of velocities. It has two contributions: a viscous part $\sim \eta/h^2$ arising because flows within an adhered cell of thickness h in the vertical z direction and no-slip at the base must in general have z -gradients on a scale h , and direct damping of flow through the kinetics of attachment and detachment of the cytoskeletal gel to the substrate. In our estimates below we retain only the viscous effect, so that ζ simply has the effect of screening the hydrodynamics at in-plane length-scales larger than h . Including attachment-detachment enhances ζ . We use circular polar coordinates r, ϕ in the plane. Since we assume axisymmetry, the radial velocity vanishes because incompressibility implies $dv_r/dr + v_r/r = 0$, and $v_r = 0$ at both the interfaces. For force balance in the region corresponding to the nucleus we have to solve the equation

$$\eta_i \frac{d^2}{dr^2} v_\phi + \frac{\eta_i}{r} \frac{d}{dr} v_\phi - \frac{\eta_i}{r^2} v_\phi = 2\zeta_i v_\phi, \quad (4)$$

where $\zeta_i = \eta_i/h^2$. The equation can be solved in terms of Bessel functions, with the constraint that v_ϕ has to be 0 at $r = 0$. Continuity of tangential stress and velocity at the cytoplasm-nucleus interface gives the requisite number of boundary conditions.

Force balance in the azimuthal direction reads

$$\frac{d}{dr} \sigma_{\phi r}^s + \frac{2\sigma_{\phi r}^s}{r} = \zeta v_\phi. \quad (5)$$

Expressing the director $\mathbf{n} = (n_\phi, n_r) = (\cos \theta, \sin \theta)$ the steady state equation (1) for the orientation field reads

$$\frac{1}{\Gamma} \frac{\delta F}{\delta \theta} = \frac{v_\phi}{r} + \lambda \cos 2\theta A_{r\phi} + \Omega_{r\phi} = (\lambda \cos 2\theta - 1) A_{r\phi} \quad (6)$$

where \mathbf{A} and $\mathbf{\Omega}$ are the symmetric and antisymmetric parts of the velocity gradient tensor.

Using (6), the $r\phi$ component of (3) can be recast as a first order differential equation for v_ϕ .

$$\frac{d}{dr}v_\phi = \frac{2W \sin 2\theta + 4\sigma_{\phi r}^s}{2\eta + \Gamma(\lambda \cos 2\theta - 1)^2} + \frac{v_\phi}{r} \quad (7)$$

Thus, we have two first order equations, (7) and (5), and one second order equation (6) to solve, which we solve numerically.

A noticeable and robust feature of the solution (inset to Fig. 5A) is the presence of a maximum in the magnitude of the velocity at some distance from the nucleus. This results from a combination of vanishing velocity at the outer boundary and the nuclear centre, and continuity of velocity and shear stress at the fluid-fluid interface. Note that our description does not include chiral effects, so that equivalent solutions with either sense of rotation are obtained.

The competition between active stresses that promote flow and orientational relaxation, that inhibits it, is contained in the dimensionless combination $\alpha = W/\zeta D \sim Wh^2/K$ [27, 31]. Accurate estimates of parameters for our system are not easy to make. The cytoskeletal active stress, W , is generally argued to be in the range 50-1000 Pa [30]. Frank constants for actin nematics appear to be 2-20 pN [32], as in ordinary thermotropic nematics. The thickness h of the spread cell in our experiments is about 1/5 of the lateral extent. For a spread cell area of $1600 \mu\text{m}^2$ we therefore estimate $h \sim 8 \mu\text{m}$. Taken together, this leads to $\alpha \sim 200$. However, if attachment-detachment contributions to ζ are included, α will be lowered substantially. From the active hydrodynamic model we know [26] that the system is quiescent for small values of this parameter. However, $\alpha = 4.9$, for which we present the results, is already sufficient to produce a spontaneous flow. Increasing α leads to increasingly complicated flows which we have only begun to explore [37]. We do not attempt a detailed comparison between the observed and the theoretical flow patterns. However, the conclusion about the maximum of the velocity being away from the nucleus rests purely upon the confining geometry, and we expect that the time and angle averaged velocity profile, measured from the experiment, will have a peak away from the nuclear boundary.

For $\alpha \gg 1$ i.e an unbounded, oriented active fluid, one expects [26] spontaneous velocity gradients of order $\frac{W}{\eta}$. In [27] and [31] it was shown that the presence of confinement on a scale h modifies the above conclusion giving a characteristic rate $\frac{W}{\eta} F(\frac{h}{\ell})$ [31] where $F(x) \rightarrow 1$ as $x \rightarrow \infty$ and $\sim x^2$ for $x \rightarrow 0$, where ℓ is the in-plane scale associated with observation. In our case, $\frac{h}{\ell}$ is 1/5. Thus, the rotation rate should be of the order of $0.1W/\eta$. Using the arguments of [30] this estimate turns out to be of the order of a few degrees/min. This is reassuringly consistent with the magnitude obtained from the experiment.

The two predictions we can make based on this simple model are that actomyosin is crucial for nuclear rotation, and that the angle and time averaged angular velocity will be maximum away from the nucleus. We perform a series of experiments to check these. In the next sections, we study the contribution of actomyosin contractility, a critical cytoplasmic regulator of nuclear prestress [38, 39], to the translational and rotational dynamics of the nucleus.

Role of actomyosin contractility on nuclear dynamics

We test the role of contractility on nuclear dynamics to validate the theoretical predictions based on active fluids with an inclusion. Actomyosin contractility was altered by treating cells fully spread on geometric patterns with low concentration of blebbistatin an inhibitor of the myosin II motor. To determine if the persistence in nuclear translation motion was dependent on contractility, the autocorrelation function (ACF), was plotted for control and blebbistatin treated cells (Figure 3A). Blebbistatin treated cells exhibited a decreased correlation time scale for translational motion (bottom inset to Figure 3A,) suggesting that actomyosin contractility is important for correlated translational movement of the nucleus. Next, the nuclear rotation angle as a function of time was calculated from the XY rotation trajectories. A typical plot of angle versus time for the nucleus on geometric pattern is shown in Supplementary Figure S3. On treatment with blebbistatin, the instantaneous angular velocity significantly decreases to $1.0^\circ/\text{min}$ when compared to control $1.6^\circ/\text{min}$ (top inset to Figure 3A and Supplementary Figure S4). To ascertain the effect of actomyosin contractility on the persistence of nuclear rotation we computed the auto-correlation of angular movement with time. Figure 3B shows plot of auto-correlation curve for control cells and cells treated with blebbistatin. Inset (below) to Figure 3B show that on perturbing actomyosin contractility, the persistence time of nuclear rotation decreases from 62 min in control to 33 min. In the next section, we study the role of actin flow patterns in regulating the nuclear dynamics.

Role of actin flow in driving nuclear dynamics

Live cell fluorescence confocal imaging was carried out to simultaneously visualize actin flow dynamics and nuclear rotation on geometric patterns. Cells were transfected with lifeact-GFP to label actin in live condition (Figure 4). Time lapse confocal imaging of actin revealed a retrograde flow and its remodeling around the nucleus (Figure 4A). To quantify the flow pattern (Supplementary Movie 6), we carried out particle image velocimetry (PIV) analysis using MatPIV. This revealed flow vectors tangential to the nuclear boundary with direction and magnitude correlated with that of the nuclear rotation as shown in Figure 4A and Supplementary Movie 7. Velocity field maps of actin flow were determined in small regions throughout the cell (Figure 4A, last panel and Supplementary Movie 8). A circulating flow, required to rotate the nucleus, is clearly seen (Figure 4A, middle panel and Supplementary Movie 6 and 7). Interestingly, upon blebbistatin treatment, inward flow of actin (Supplementary Movie 9-11), presumably driven by treadmilling, was not significantly affected. However, the azimuthal speed can be seen (Figure 4B) to decrease substantially, despite some scatter in the data. The circulation of flow around the nucleus was lost concurrent with the loss of nuclear rotation (Figure 4B, middle panel and Supplementary Movie 10).

Further, we plot in Figure 5A and B, the angle averaged azimuthal velocity v_ϕ , with and without blebbistatin respectively, inferred from PIV as a function of radial distance from the centre of the nucleus. For comparison we also show the radial velocity, v_r (Figure 5 C and D). Note that the graphs start from the edge of the nucleus. As predicted from the theory, the azimuthal velocity peaks away from the nuclear boundary in the control cells. In blebbistatin treated cells, by contrast, the velocity is 0, leading to the loss of nuclear rotation. However, v_r is small in both cases, albeit with slightly larger fluctuations in the presence of blebbistatin. In addition, time lapse imaging of microtubules labeled with tau-EGFP show that the microtubule organizing centre (MTOC) undergoes translation dynamics while the nucleus exhibits both translational and rotational dynamics (Supplementary Figure S5). The orientation and arrangement of microtubules showed a cage like structure around the nucleus (Supplementary Movie 12). This caging mechanism might help keep the nucleus relatively localized, thus enhancing the rotational effects of the torque generated by the actin flow. We also visualized the endoplasmic reticulum (ER) to assess its role in nuclear dynamics. Since ER is contiguous with the nuclear envelope, it could either stretch or undergo continuous remodeling as the nucleus rotates. Live cell imaging of ER, during nuclear rotation, showed dynamic remodeling suggesting a minor role for ER in nuclear rotation and reversals (Supplementary Figure S6).

CONCLUSION

Our results show that geometric constraints are critical in determining the rotational dynamics of the cell nucleus. While a number of components including cytoskeleton and motor proteins have been implicated to drive nuclear dynamics in migrating cells, our results on single cells confined to specific geometries suggest a role for actomyosin contractility. Square, circular, and triangular pads support mainly rotational motion, while long narrow geometries restrict it. The shape of the confining geometry further determines the magnitude of nuclear rotation; a relatively faster rotating nucleus is seen on circular pattern than on squares and triangles. We offer a simple theoretical explanation for the rotation in which the nucleus is modelled as a nearly rigid inclusion in the cytoplasm treated as a fluid containing filaments endowed with intrinsic stresses. The result is an angular velocity profile with a maximum at a radial position intermediate between the nucleus and the cell periphery, as observed in the experiments, and nonzero at the nuclear surface, corresponding to nuclear rotation. The predicted magnitude of the rotation rate based on plausible estimates of material parameters are also consistent with the measurements. That blebbistatin treatment greatly suppresses the flow lends support to our proposed mechanism. The question arises why nuclei are not universally observed to rotate in cells under normal conditions. At least two mechanisms could contribute to suppressing the generic instability that leads to circulating flows. One, in the absence of a rigid geometry, the cell boundary is free to change shape. This would disrupt the imposed boundary orientation of the filaments, and hence the orderly pattern of active stresses needed to drive a coherent flow. Two, the apical actin fibres [44], absent in square and circular geometries, present to some extent in triangular geometries, and very well formed in elongated geometries, bear down on and thus enhance the friction on the nucleus, suppressing its motion.

Collectively, our results highlight the importance of both cell geometric constraints and actomyosin contractility in determining nuclear homeostatic balance. A number of experiments have shown that alterations in cell geometry affect gene expression programs [40] and cell cycle time [41], and lead to a switching of cell fates towards apoptosis or proliferation [42, 43]. We hope our work leads to a search for nuclear rotation in a wider range of systems and settings, whether such rotation has biologically significant consequences, and a deeper understanding of how the cell normally suppresses such effects.

A.K., M.S., and G.V.S. thank the Mechanobiology Institute (MBI) at the National University of Singapore (NUS) for funding and MBI facility. A.M. thanks TCIS, TIFR Hyderabad for support and hospitality, and S.R. acknowledges a J.C. Bose fellowship

-
- [1] Dahl KN, Ribeiro AJ, and Lammerding J, *Nuclear shape, mechanics, and mechanotransduction*, Circ Res 102(11):1307-1318 (2008).
 - [2] Crisp M, et al. *Coupling of the nucleus and cytoplasm: role of the LINC complex*, J Cell Biol 172(1):41-53 (2006).
 - [3] Haque F, et al. *SUN1 interacts with nuclear lamin A and cytoplasmic nesprins to provide a physical connection between the nuclear lamina and the cytoskeleton*, Mol Cell Biol 26(10):3738-3751 (2006).
 - [4] Houben F, Ramaekers FC, Snoeckx LH, and Broers JL, *Role of nuclear lamina- cytoskeleton interactions in the maintenance of cellular strength*, Biochim Biophys Acta (2006).
 - [5] Wang N, Tytell JD, and Ingber DE *Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus*, Nat Rev Mol Cell Biol 10(1):75-82 (2009).
 - [6] Takiguchi K *Heavy Meromyosin Induces Sliding Movements between Antiparallel Actin Filaments* J Biochem. 109: 520-527 (1991)
 - [7] King MC, Drivas TG, and Blobel G *A network of nuclear envelope membrane proteins linking centromeres to microtubules*, Cell 134(3):427-438 (2008).
 - [8] Theriot JA, *The polymerization motor*, Traffic 1(1):19-28 (2000).
 - [9] Tzur YB, Wilson KL, and Gruenbaum Y, *SUN-domain proteins: 'Velcro' that links the nucleoskeleton to the cytoskeleton*, Nat Rev Mol Cell Biol 7(10):782-788 (2006).
 - [10] Zhang Q, et al. *Nesprin-2 is a multi-isomeric protein that binds lamin and emerin at the nuclear envelope and forms a subcellular network in skeletal muscle*, J Cell Sci 118(Pt 4):673-687 (2005).
 - [11] Brosig M, Ferralli J, Gelman L, Chiquet M, and Chiquet-Ehrismann R, *Interfering with the connection between the nucleus and the cytoskeleton affects nuclear rotation, mechanotransduction and myogenesis*, Int J Biochem Cell Biol 42(10):1717-1728 (2010).
 - [12] Lee JS, Chang MI, Tseng Y, and Wirtz D, *Cdc42 mediates nucleus movement and MTOC polarization in Swiss 3T3 fibroblasts under mechanical shear stress*, Mol Biol Cell 16(2):871-880 (2005).
 - [13] Levy JR and Holzbaur EL, *Dynein drives nuclear rotation during forward progression of motile fibroblasts*, J Cell Sci 121(Pt 19):3187-3195 (2008).
 - [14] Luxton GW, Gomes ER, Folker ES, Vintinner E, and Gundersen GG, *Linear arrays of nuclear envelope proteins harness retrograde actin flow for nuclear movement*, Science 329(5994):956-959 (2010).
 - [15] Reinsch S and Gonczy P, *Mechanisms of nuclear positioning*, J Cell Sci 111 (Pt 16):2283-2295 (1998).
 - [16] Starr DA *Communication between the cytoskeleton and the nuclear envelope to position the nucleus*, Mol Biosyst 3(9):583-589 (2007).
 - [17] Wu J, Lee KC, Dickinson RB, and Lele TP, *How dynein and microtubules rotate the nucleus*, J Cell Physiol 226(10):2666-2674 (2011).
 - [18] Hagan I and Yanagida M, *Evidence for cell cycle-specific, spindle pole body-mediated, nuclear positioning in the fission yeast Schizosaccharomyces pombe*, J Cell Sci 110 (Pt 16):1851-1866 (1997).
 - [19] Marchetti et.al, *Soft active matter* Rev. Mod. Phys., (in press); arXiv:1207.2929
 - [20] Ramaswamy S, *The Mechanics and Statistics of Active Matter*, Annu. Rev. Condens. Matt. Phys. 1, 323-345 (2010)
 - [21] Joanny J-F, Jülicher F, Kruse K, and Prost J *Hydrodynamic Theory for Multi-Component Active Polar Gels* New J. Phys. 9, 422 (2007)
 - [22] Jülicher F et.al. *Active Behavior of the Cytoskeleton* Phys. Rep. 3, 449, (2007)
 - [23] Toner J, Tu Y, and Ramaswamy S, *Hydrodynamics and phases of flocks*, Ann. Phys. 318, 170 (2005)
 - [24] S. Fürthauer et.al., *The TaylorCouette motor: Spontaneous Flows of Active Polar Fluids Between Two Coaxial Cylinders* New. J. Phys. 14, 023001 (2012)
 - [25] Woodhouse FG and Goldstein RE, *Spontaneous Circulation of Confined Active Suspensions* Phys. Rev. Lett. 109, 168105 (2012)
 - [26] Simha RA and Ramaswamy S *Hydrodynamic fluctuations and instabilities in ordered suspensions of self-propelled particles*, Phys Rev Lett 89, 058101 (2002)
 - [27] Voituriez R, Joanny J-F, and Prost J, *Spontaneous flow transition in active polar films*, EuroPhys. Lett. 70 404 (2005)
 - [28] Kruse K et al. *Asters, Vortices, and Rotating Spirals in Active Gels of Polar Filaments* Phys. Rev. Lett. 92, 078101 (2004)
 - [29] Liverpool TB and Marchetti C *Organization and instabilities of active polar filaments*, Phys. Rev. Lett. 90, 138102 (2003).
 - [30] Joanny JF and Prost J *Active gels as a description of the actin-myosin cytoskeleton* HFSP Journal, 3(2):94-104 (2009).
 - [31] Ramaswamy S and Rao M *Active-filament hydrodynamics: instabilities, boundary conditions and rheology* New J. Phys. 9, 423 (2007)
 - [32] Lai GH et.al., *Self-Organized Gels in DNA/F-Actin Mixtures without Crosslinkers: Networks of Induced Nematic Domains with Tunable Density* Phys. Rev. Lett. 101, 218303 (2008)
 - [33] de Gennes PG, and Prost J, *The Physics of Liquid Crystals, Second Edition*, Clarendon Press(1993).
 - [34] Stark H and Lubensky TC *Poisson-bracket approach to the dynamics of nematic liquid crystals*, Phys. Rev. E 67, 061709

- (2003).
- [35] Martin PC, Parodi O, and Pershan PS, *Unified hydrodynamic theory for crystals, liquid crystals, and normal fluids*, Phys. Rev. A 6 , 2401 (1972).
 - [36] Landau L, and Lifshitz EM, *Theory of Elasticity, Third Edition: Volume 7 (Theoretical Physics)*, Butterworth-Heinemann(1986).
 - [37] Maitra A, and Ramaswamy S,(*To be published*)
 - [38] Mazumder A and Shivashankar GV, *Gold-nanoparticle-assisted laser perturbation of chromatin assembly reveals unusual aspects of nuclear architecture within living cells*, Biophys J 93(6):2209-2216 (2007).
 - [39] Mazumder A and Shivashankar GV, *Emergence of a prestressed eukaryotic nucleus during cellular differentiation and development*, J R Soc Interface 7 Suppl 3:S321-330 (2010).
 - [40] Kilian KA, Bugarija B, Lahn BT, and Mrksich M, *Geometric cues for directing the differentiation of mesenchymal stem cells*, Proc. Natl. Acad. Sci. USA 107(11):4872-4877 (2010).
 - [41] Ingber DE, et al. *Cellular tensegrity: exploring how mechanical changes in the cytoskeleton regulate cell growth, migration, and tissue pattern during morphogenesis*, Int Rev Cytol 150:173-224 (1994).
 - [42] Gray DS, et al. *Engineering amount of cell-cell contact demonstrates biphasic proliferative regulation through RhoA and the actin cytoskeleton*, Exp Cell Res 314(15):2846-2854 (2008).
 - [43] Sun Y, Chen CS, and Fu J *Forcing stem cells to behave: a biophysical perspective of the cellular microenvironment*, Annu Rev Biophys 41:519-542 (2012).
 - [44] Li Q, Kumar A and Shivashankar GV, *Cellular geometry mediated apical stress fibers dynamically couples nucleus to focal adhesion* (under review).

FIGURES

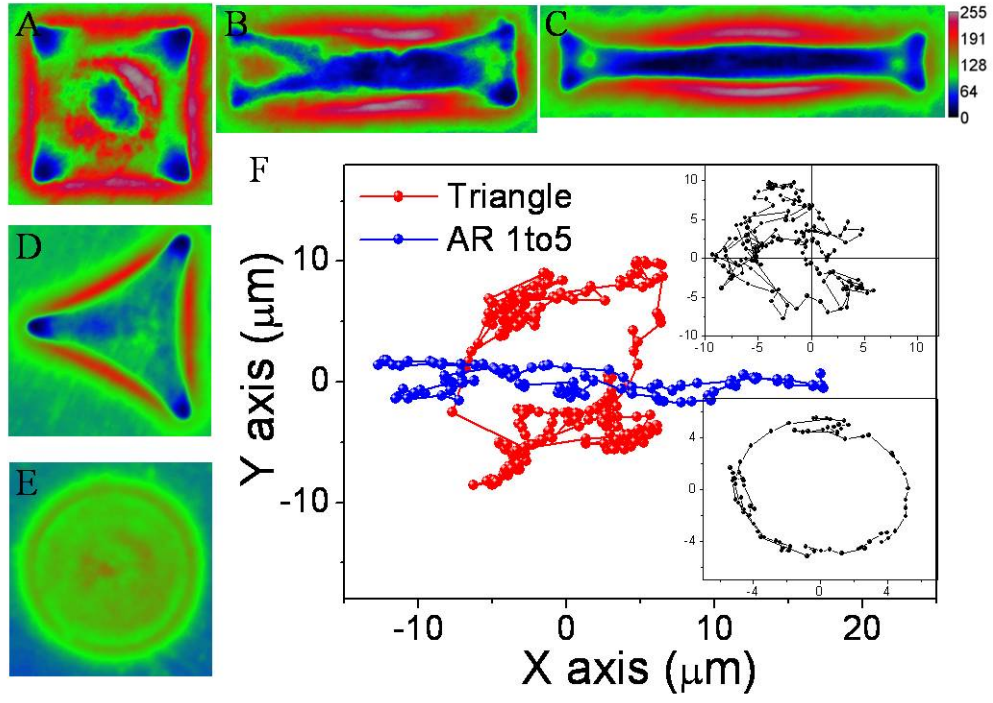


FIG. 1: Geometric constraints impinge on translational and rotational dynamics of the cell nucleus. (A-E) Colour -coded kymograph showing average intensity projection of time lapse phase contrast images for various shapes: rectangles of aspect ratio 1:1 (A), 1:3 (B) and 1:5 (C), triangle (D) and circle (E). (F) Typical XY trajectory of nucleus on triangle (red) and rectangle of aspect ratio 1:5. Inset: a typical translational (top) and rotational (below) trajectory of nucleus on triangular pattern.

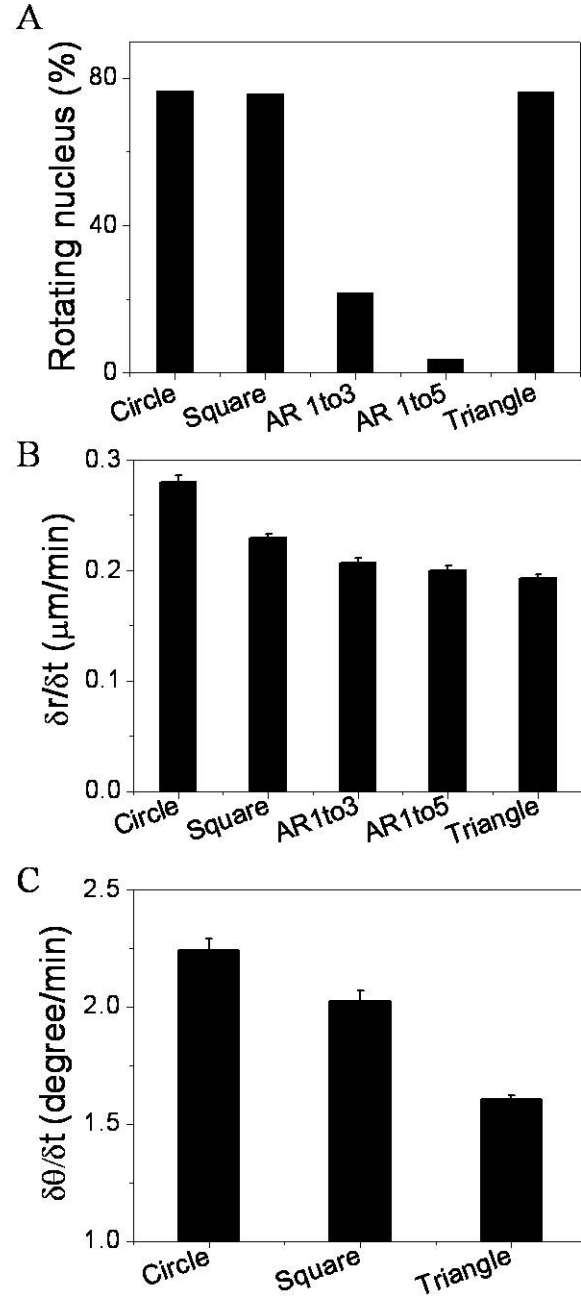


FIG. 2: Quantification of the translational and rotational dynamics of the nucleus. (A) Fraction of nucleus which rotate on circles, squares, triangles, and rectangles of aspect ratio 1:3 and 1:5. (B) Instantaneous linear velocity of the nucleus on these patterns (C) Instantaneous angular velocity of the nucleus on circles, squares, and triangles. Error bars are SEM.

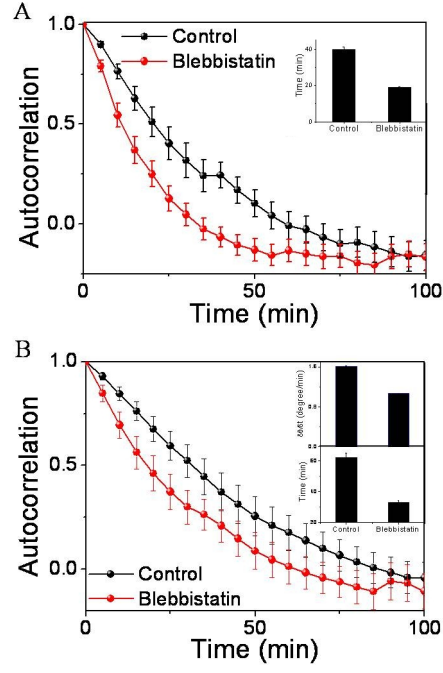


FIG. 3: Actin contractility affects persistence of both translational and rotational movement of the nucleus. (A) Mean autocorrelation curve for nuclear displacement in control (black) and blebbistatin (red) treated cells. Inset: Persistence time of translation for the two cases obtained by fitting the mean autocorrelation curves with single exponential decay function. (B) Mean autocorrelation curve for angular fluctuation for control (black) and blebbistatin (red) treated cells. Insets: Instantaneous angular velocity (top) and angular time scale (bottom) for the two cases. Error bars are SEM.

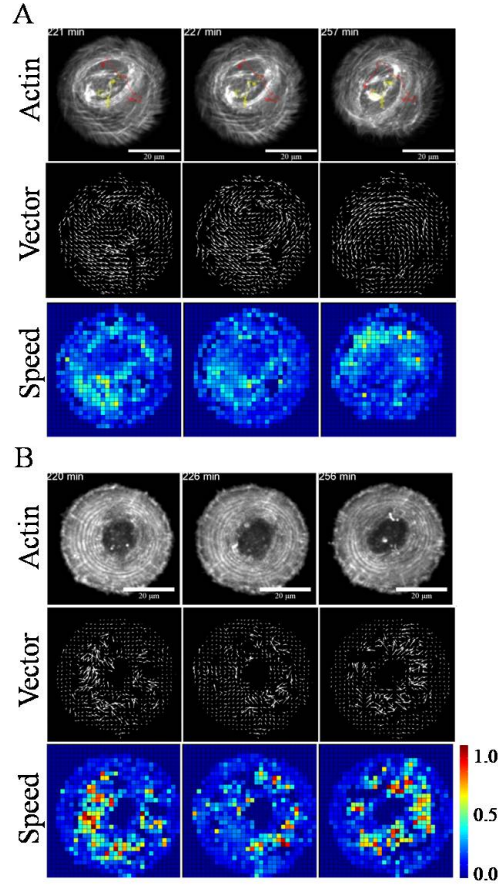


FIG. 4: Visualization of actin flow patterns during nuclear rotation in both control and blebbistatin treated cells plated on circular geometry. (A) Top panel: Tracks of two nucleoli (red and yellow) showing both translational and rotational dynamics. Scale bar = $20\mu\text{m}$. Corresponding actin flow vectors (middle panel) and speed (last panel) was determined by particle image velocimetry (PIV) analysis using MatPIV for control (A) and blebbistatin (B) treated cells. The flow vectors have been scaled to 2 times for better visibility. Color code: 0.0 - 1.03 $\mu\text{m}/\text{min}$.

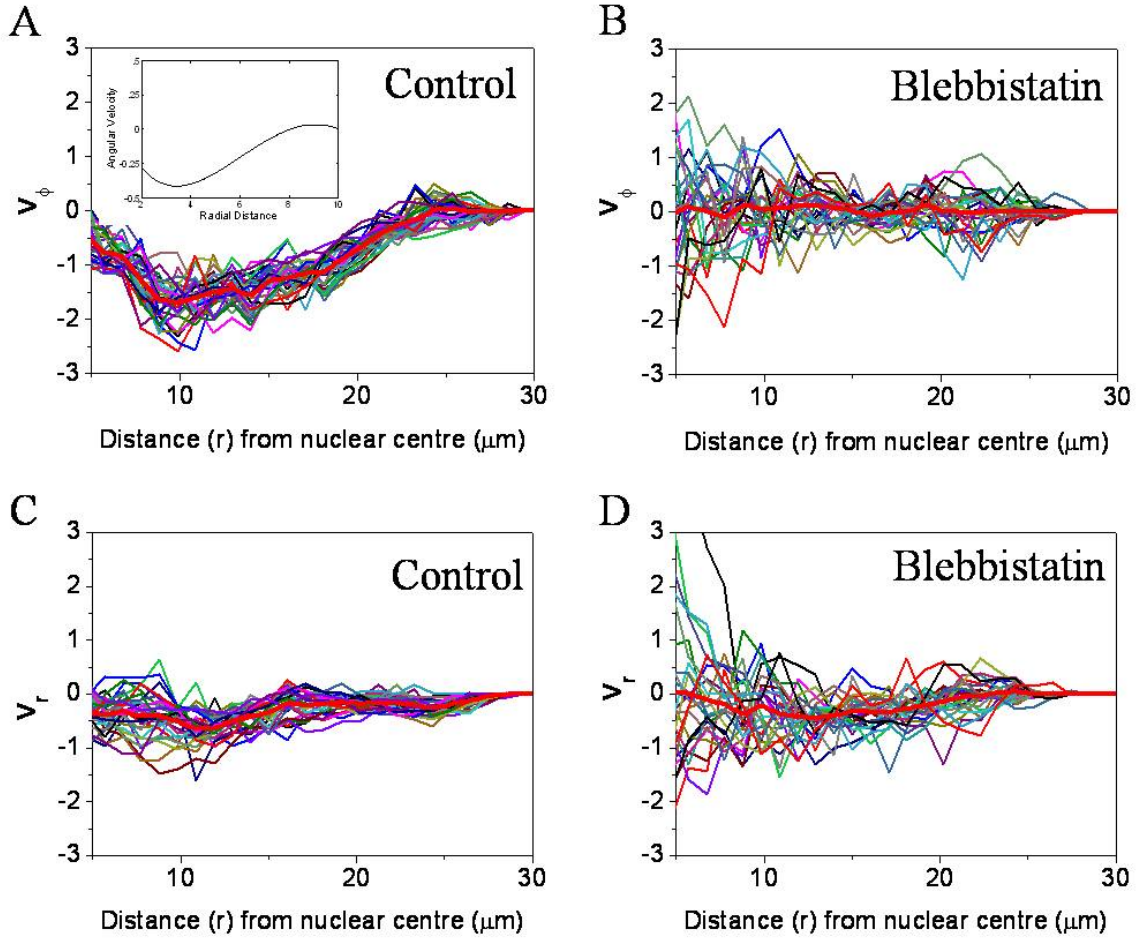


FIG. 5: Azimuthal and Radial velocity of actin flow plated on circular geometry. Plot of v_ϕ and v_r from velocity vectors of actin flow for control (A) and (C); and blebbistatin treated cells (B) and (D). Each color represents single time point for cells. Thick red curve is the mean of various such realizations (30 for control and 25 for blebbistatin treated cells). Inset to 5A: A typical angular velocity vs. radial distance curve obtained by solving the equations (5), (6), and (7).

SUPPLEMENTARY INFORMATION

Supplementary movies

[Movies are available by e-mail to G.V. Shivashankar]

Movie 1: Phase contrast images of the cell on circular geometry. Images were acquired every 5 minute. Scale bar= 20 micron.

Movie 2: Phase contrast images of the cell on square geometry. Images were acquired every 5 minute. Scale bar= 20 micron.

Movie 3: Phase contrast images of the cell on rectangular geometry of aspect ratio 1:3. Images were acquired every 5 minute. Scale bar= 20 micron.

Movie 4: Phase contrast images of the cell on rectangular geometry of aspect ratio 1:5. Images were acquired every 5 minute. Scale bar= 20 micron.

Movie 5: Phase contrast images of the cell on triangular geometry. Images were acquired every 5 minute. Scale bar= 20 micron.

Movie 6: Actin flow pattern on circular geometry.

Movie 7: Actin flow vectors on circular geometry.

Movie 8: Actin velocity field maps on circular geometry.

Movie 9: Actin flow pattern in cells treated with blebbistatin on circular geometry.

Movie 10: Actin flow vectors in cells treated with blebbistatin on circular geometry.

Movie 11: Actin velocity field maps in cells treated with blebbistatin on circular geometry.

Movie 12: 3D image of microtubule constructed from high resolution confocal images.

Supplementary figures

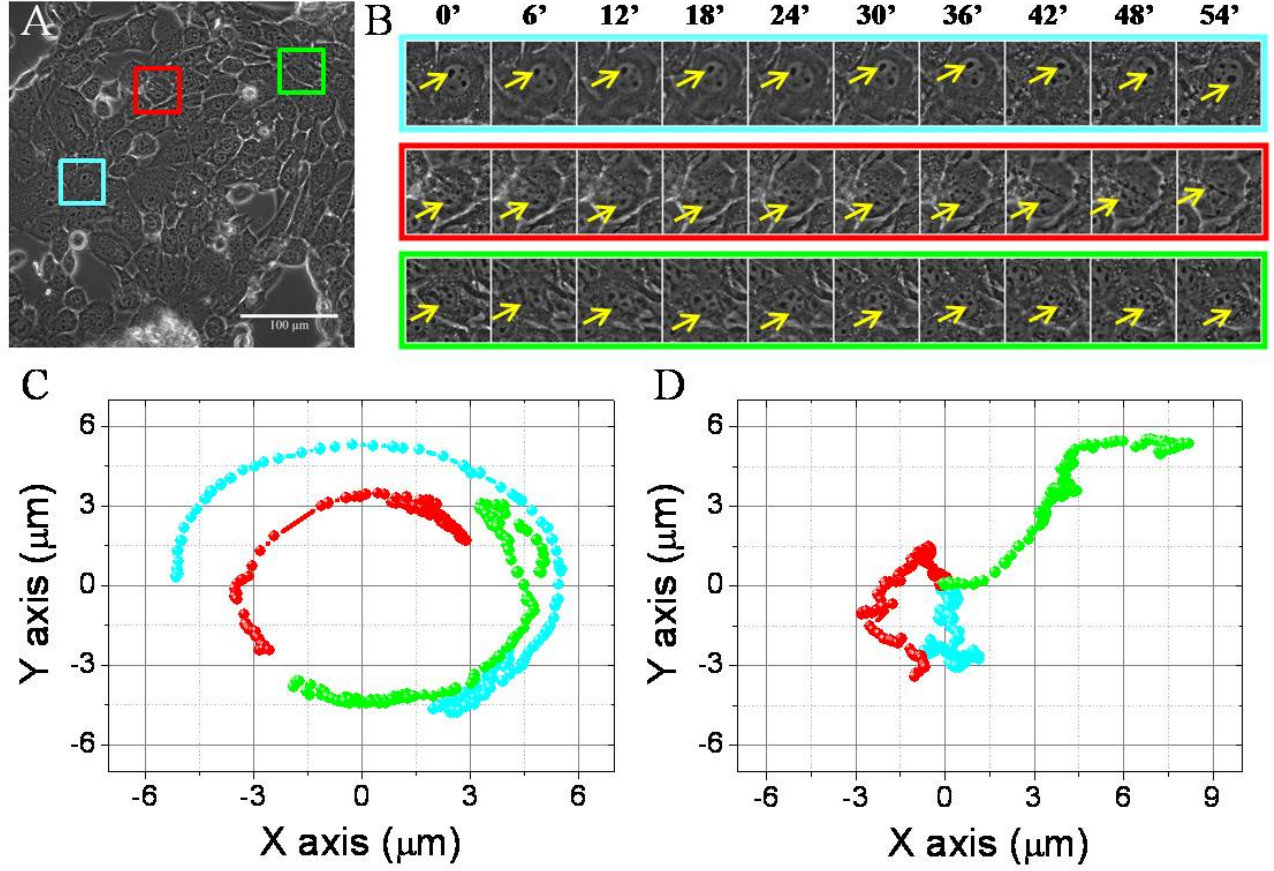


FIG. S1: Nuclear rotation in a monolayer of NIH3T3 cells. (A) Representative DIC images of NIH3T3 cells growing as a monolayer on glass bottom culture dishes. Scalebar = 100 μm . (B) Time lapse images of single cells from different regions (cyan, red and green rectangles shown in (A)) of the monolayer. Time points are indicated at the top of each image. Plot of rotational (C) and translational (D) movement for the three cells shown in (B).

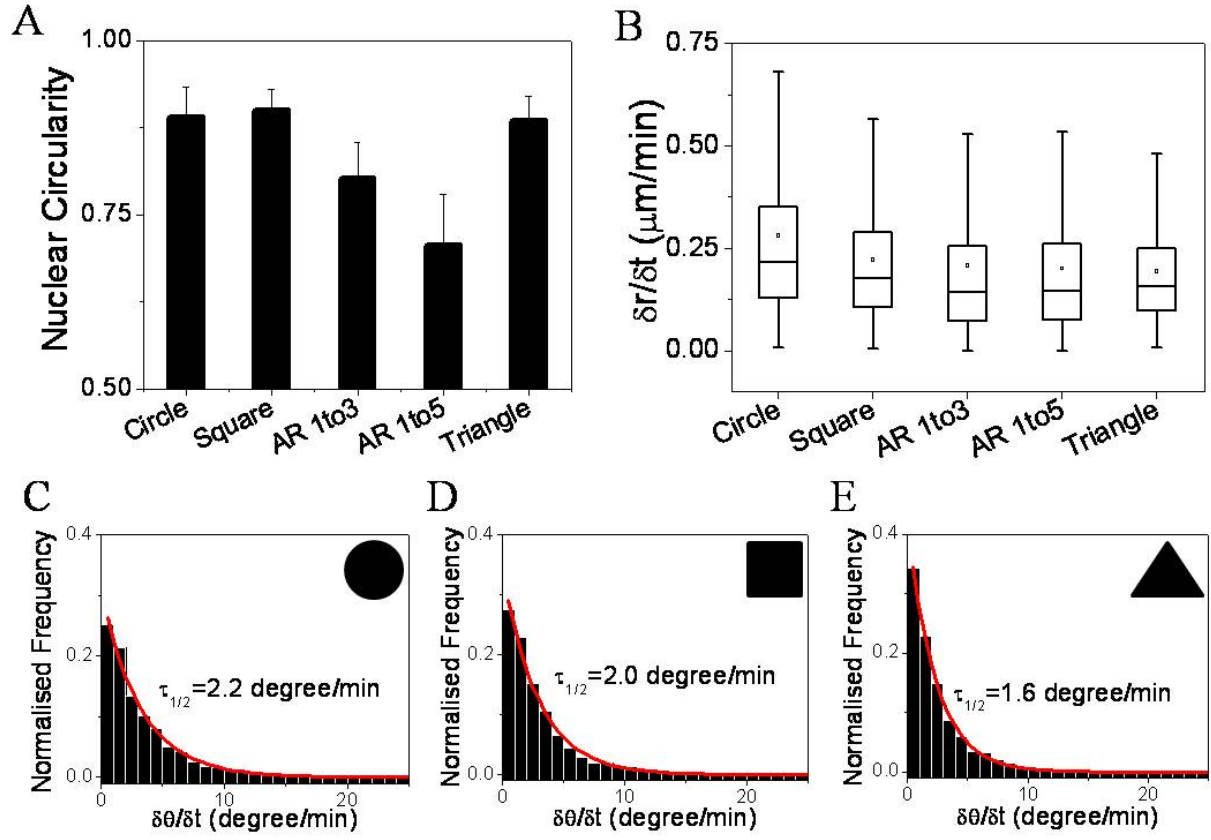


FIG. S2: (A) Nuclear circularity calculated from phase contrast image of the cell on various patterns. (B) Box plot of instantaneous linear velocity showing mean (small box), median (horizontal line within the box), 25th to 75th percentile (box range) and 5th to 95th percentile (vertical line) of nuclear movement on circles, squares, triangles, and rectangles of aspect ratio 1:3 and 1:5. (C) to (E) Histograms of instantaneous angular velocity (vertical box) and corresponding single exponential decay fit (red line) for circles, squares and triangles. The corresponding instantaneous angular velocities are quoted in the plot.

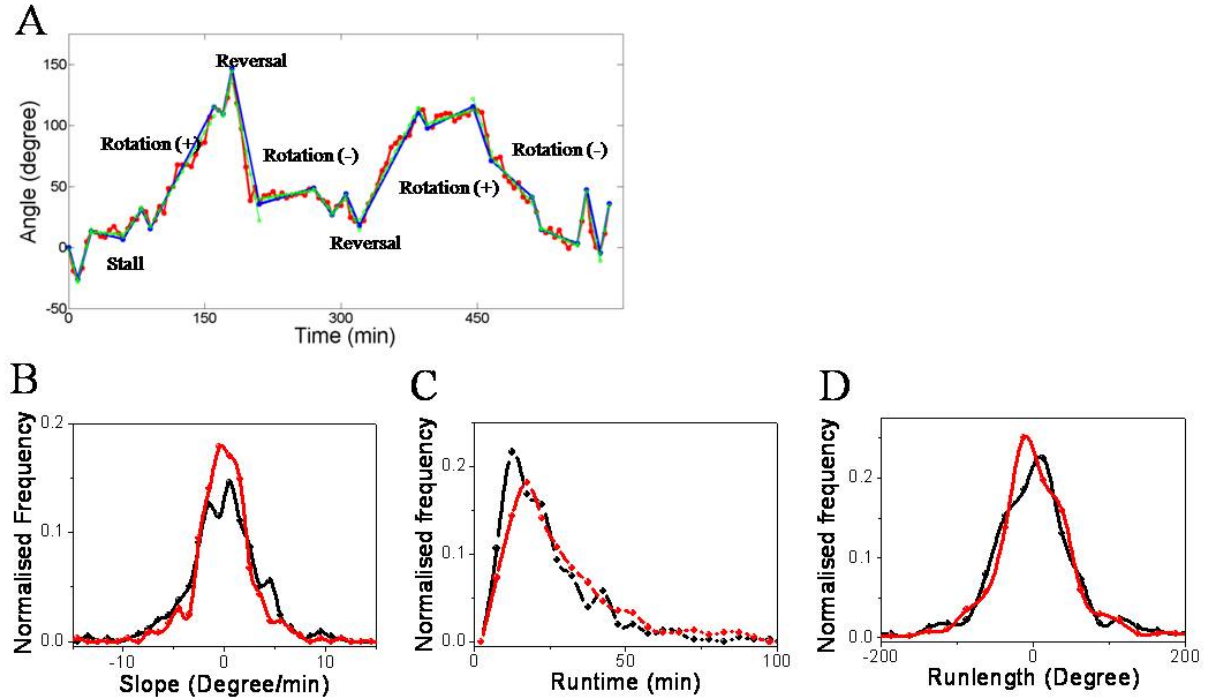


FIG. S3: (A) A typical angle versus time plot depicting various regimes (rotation, reversal and stalling) of rotational motion (red). Blue line joins the vertices obtained from Ramer DouglasPeucker algorithm for piecewise linear function which reduces the number of points from the curve depending on tolerance. Linear fit to these regimes obtained from the above algorithm (green). Histogram of slopes (B) runtime (C) and runlength (D) for various regimes during rotation for multiple cells on triangular pattern (control-black) and blebbistatin treated cells (red). To further analyse nuclear rotation dynamics, the angle versus time was divided into different regimes: rotation, stalls and reversals. Linear fits were generated for the three respective regimes to compute the average angular velocity, runtime and runlength and their distributions were found to be similar for both control and blebbistatin treated cells. Although the typical nuclear rotational trajectory shows the three regimes, the ratio of total runtime in positive and negative directions is 1.8.

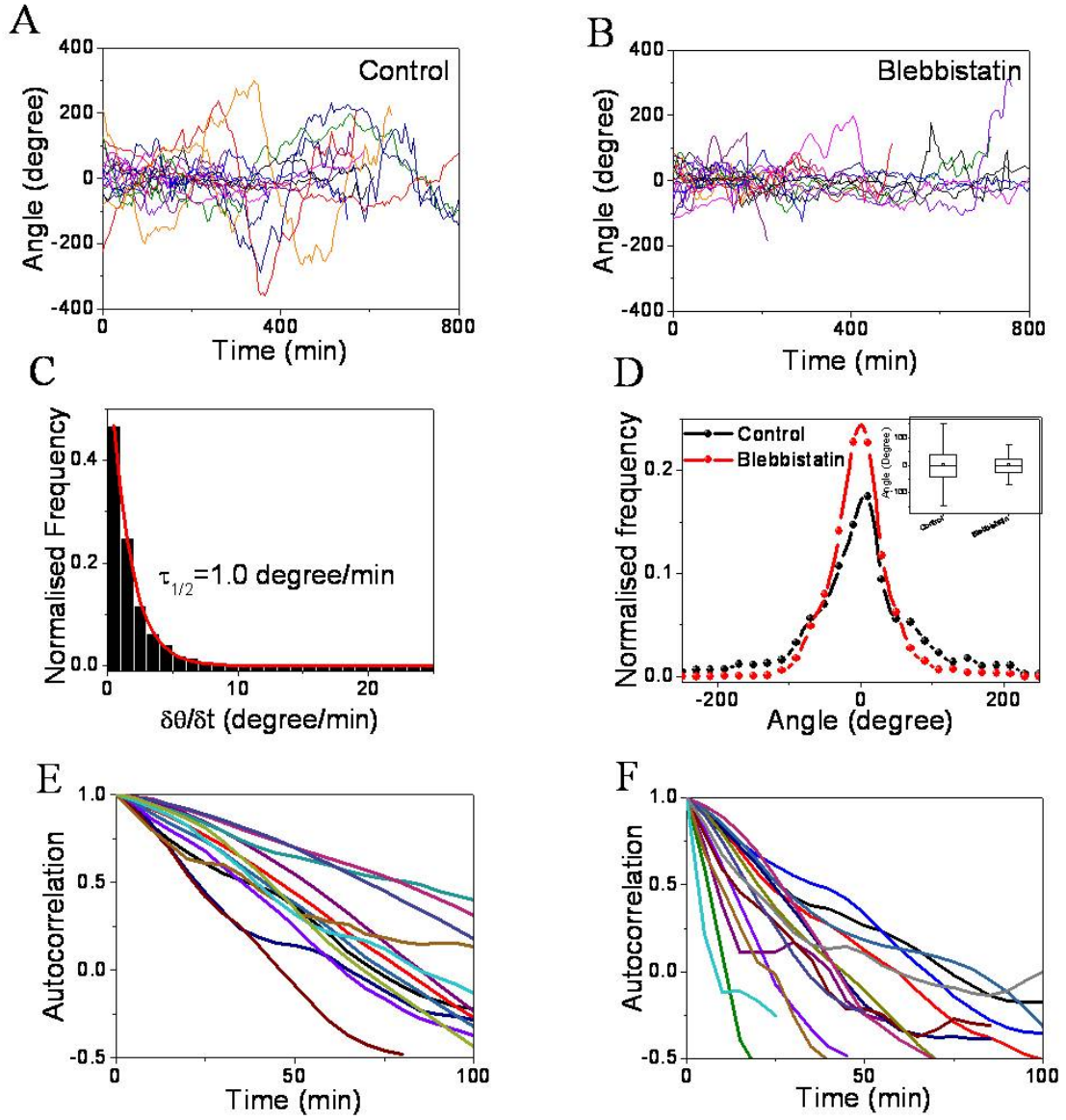


FIG. S4: Plot showing angle versus time curves obtained from nuclear rotation trajectory for control (A) and blebbistatin (B) treated cells on triangular geometry. (C) Histograms of instantaneous angular velocity (vertical box) and corresponding single exponential decay fit (red line) for blebbistatin treated cells. (D) Histogram of the rotation of the nucleus in both control and drug treated cases. Inset: Box plot of angle showing decreased width in the drug treated case. Angular autocorrelation curves for control (E) and Blebbistatin treated cells (F). The correlation timescale was determined by fitting individual curves to a single exponential decay. Each color represents different cells.

A

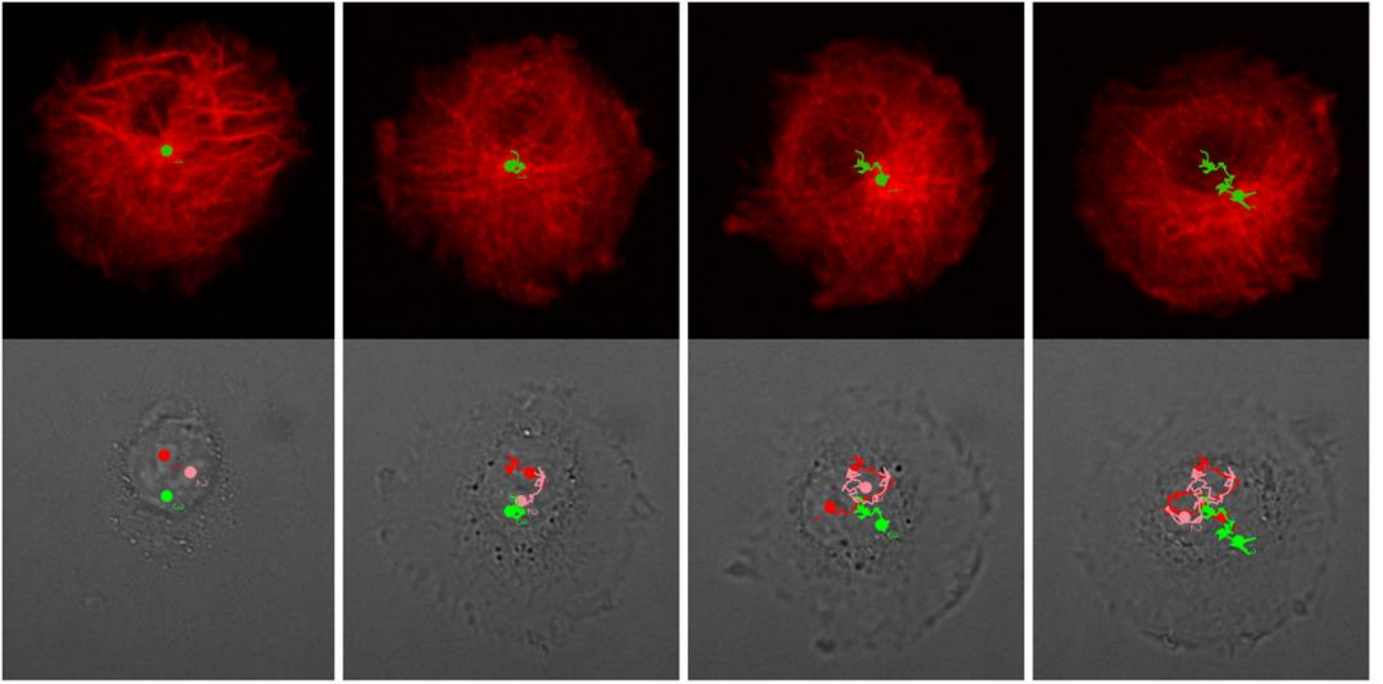


FIG. S5: Movement of microtubule organization centre (MTOC) labeled using tau-RFP. Tracks of MTOC (green), movement of two nucleoli (red and pink) overlaid on fluorescence and DIC images.

A

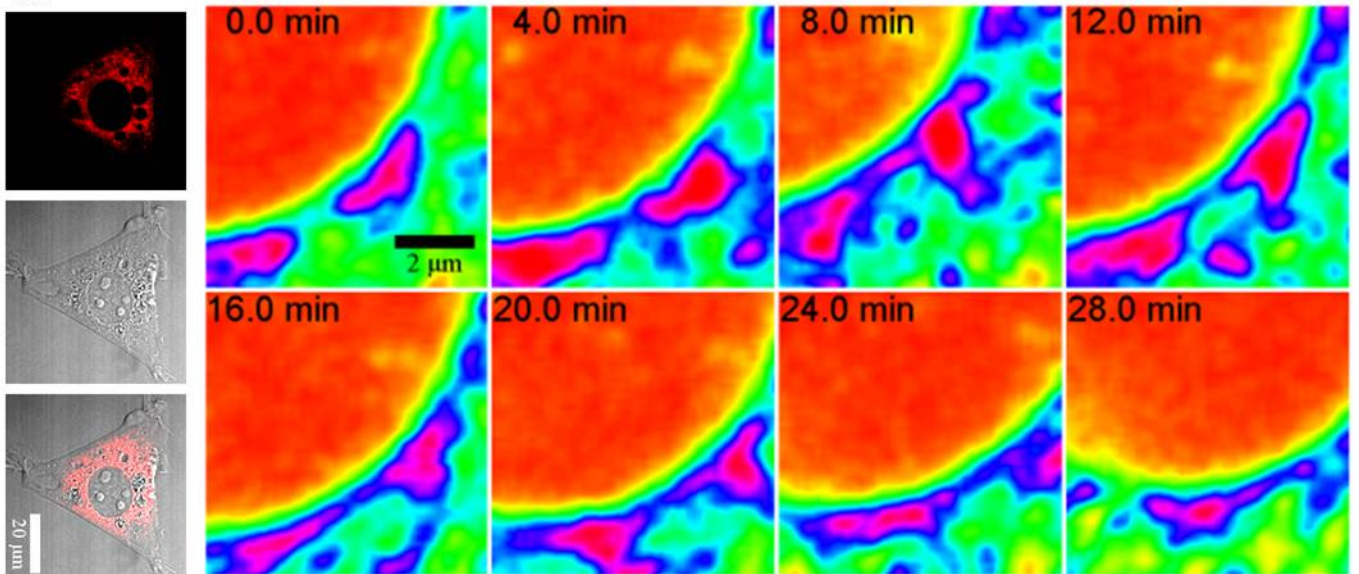


FIG. S6: Endoplasmic reticulum (ER) labeled using dsRED ER. Panel of images show reorganization of ER, near the nucleus, with time.